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Original Article

The association between the IL-4, ADRβ2 and ADAM 33 gene polymorphisms and asthma in the Taiwanese population☆

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Abstract

Background: The association between genetic polymorphisms of ADRβ2, IL-4, and ADAM3 and asthma remains undetermined. Furthermore, it is not clear as to whether these three gene polymorphisms combined produce a correlative increase in asthmatic risk.
Methods: A total of 476 asthmatic patients and 115 healthy volunteers were enrolled. Single nucleotide polymorphisms (SNPs) of the C-589T IL-4 promoter (rs2243250), the ADRβ2 gene [at nucleic acid 46 (rs1042713) and 79 (rs1042714)] and the ADAM33 gene [at rs2280091 (T1), rs3918396 (S1), rs3918391 (D1), and rs615436] were analyzed. Pulmonary function tests, methacholine challenge tests, total IgE, specific IgE for inhalant allergens and total eosinophil counts were assessed.
Results: The T allele for the IL-4 gene promoter (C-589T locus), the C allele frequency for ADRβ2 (Gln27Glu locus), and the A allele for the ADAM33 gene (rs2280091, T1) were higher in asthma patients than in normal individuals. In asthmatic patients, the A allele of ADAM33 (rs2280091) was associated with higher eosinophil count and increased hyperresponsiveness compared to the C allele. Combination of the three risk genes SNP had an additive effect on the risk of asthma (OR: 3.46; CI: 1.51−7.93) and increased the diagnostic sensitivity and specificity of asthma.
Conclusion: Genetic polymorphism in the IL-4 promoter, ADRβ2, and ADAM33 is associated with asthma. Furthermore, ADAM33 genetic polymorphism modifies the phenotype of asthma in atopy and hyperresponsiveness. Asthma is a complex polygenic disease, and combinations of polymorphisms of various genes have an additive effect on the risk of asthma.

Keywords: ADAM33; asthma; β2 adrenergic receptor; IL-4; single nucleotide polymorphism (SNP)

1. Introduction

Asthma is characterized by intermittent airway obstruction and respiratory symptoms that are related to chronic airway inflammation and remodeling.[1−3]

Multiple genes may be involved in the pathogenesis of asthma,[4,5] and different genes may be involved in different ethnic groups. Previous studies have used genetic linkage techniques to identify human chromosome 5q31-33 as a region likely to contain genes related to asthma,[6] elevated serum

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immunoglobulin E (IgE) levels[7,8] and airway hyper-responsiveness (AHR).[9] Among the candidate genes in this region is the gene encoding interleukin-4 (IL-4) and the β2-adrenergic receptor (ADRβ2). Furthermore, a positional cloning strategy has recently identified a disintegrin and metalloprotease 33 (ADAM33) in chromosome 20p13 as a susceptibility factor of asthma.[10]

A unique function of IL-4 is to promote differentiation of Th2, and it therefore acts at a proximal and critical site in the allergic response. IL-4 is critical to the synthesis of IgE by B-lymphocytes and is also involved in eosinophil recruitment to the airways.[11] Genetic variants of the promoter region of the IL-4 gene[12] have been related to elevated levels of serum IgE. Because IL-4 stimulation can influence mast cell responsiveness to IgE-mediated signaling,[13] and genetic variants in the IL-4 promoter have the capacity to modify IL-4 gene transcription, these sequence variants might be able to modify asthma. Previous studies have shown an association between IL-4 promoter polymorphism and asthma.[14–17]

Beta-2-adrenergic receptor (ADRβ2) mediate the actions of catecholamines which modulate the smooth muscle of the respiratory tree to keep airway patent. The ADRβ2 gene contains several common genetic variations that affect gene expression and receptor function *in vitro*.[18,19] The Gly16 allele of the ADRβ2 gene was correlated with more severe asthma[20,21] characterized by corticosteroid use and nocturnal symptoms,[22–24] whereas individuals homozygous for Arg16 had lower lung function.[25] The Glu27 allele of ADRβ2 gene was associated with a decrease in airway responses,[26–28] whereas Gly16 was associated with asthma susceptibility.[23,29,30] The results of ADRβ2 genetic polymorphism association studies in asthma were inconsistent.[31–33]

A disintegrin and metalloprotease 33 (ADAM33) is abundantly expressed in smooth muscle and subepithelial fibroblasts.[34,35] Expression within the structural cells of the airway wall is consistent with the hypothesis that ADAM33 is involved in airway hyper-responsiveness as an asthma-linked phenotype.[36] Recent studies have shown that the association of ADAM33 polymorphisms with asthma and related phenotypes was found in four unique asthma populations (African–American, US white, US Hispanic and Dutch white),[37] German,[38] Japanese[39] and Korean.[40] However, no association was noted in Latino populations (Puerto Rican and Mexican),[41] the North American population of childhood asthma patients,[42] German,[43] or Chinese.[44]

IL-4, ADRβ2, and ADAM33 play different roles in the pathogenesis of asthma; therefore, we propose that these three genes might be candidate genes of asthma in the Taiwanese population and that combining these genetic polymorphisms might increase the risk of asthma. To address this issue, a prospective case control study approach was used to attempt to find an association between a genetic variant of these genes and the presence of either asthma or one of the specific phenotypes of asthma. Furthermore, we attempt to ascertain whether, when combined, these genetic polymorphisms have an additive effect on the risk of asthma.

2. Methods

2.1. Study group

Asthmatic patients were enrolled based on clear-cut diagnostic criteria and were regularly followed-up in the outpatient clinic of Taipei Veterans General Hospital in Taiwan from 2000 to 2006. A total of 476 patients with asthma (age: 46 ± 20; 245 male, 231 female; FEV_1% predict: 82.6 ± 44.9%; eosinophils: 251 ± 267.3 cells/mm^3; IgE: 381.8 ± 1348.1 kU/ml; 284 atopy and 192 non-atopy) were recruited. A total of 115 healthy individuals (age: 44 ± 17; 62 male, 53 female) without any history of asthma, atopy and chronic cough or symptoms of shortness of breath were enrolled as controls. However, among the same studied population, a different number of patients were recruited to undertake this various genetic study, there are C-589T IL-4 promoter (452 asthma, 106 control), ADRβ2 gene (476 asthma, 115 control) and ADAM33 gene (382 asthma, 115 control). The hospital review board for human studies approved this study protocol, and informed consent was obtained from each participant obtained prior to participation. To be eligible for inclusion in this study, asthmatic patients were required to have all of the following characteristics defined by the guideline of the Global Initiative for Asthma (GINA)[1]: (1) at least two symptoms consistent with asthma (cough, wheeze, and dyspnea); (2) either a positive bronchial hyperresponsiveness (AHR) or a positive bronchodilator test, defined as a ≥15% increase in baseline FEV_1 after bronchodilator use; and (3) absence of other pulmonary disorders.

In asthmatic groups, pulmonary function tests, bronchodilator or methacholine bronchial provocation tests were performed to confirm airway obstruction and airway hyperreactivity, respectively. The pulmonary functions were assessed using an Automated Body Plethysmograph (6200 Autobox DL, SensorMedics Corporation, Yorba Linda, CA, USA) according to the guidelines of the American Thoracic Society.[45]

2.2. AHR was measured by bronchial challenge with methacholine

A bronchial provocation test using methacholine was conducted on 345 asthmatic patients using the dosimeter method. A total of 131 asthmatic patients did not receive this test due to the presence of moderate obstructive ventilatory impairment on pulmonary function testing, but they all had a positive bronchodilator test defined by a ≥15% increase in baseline FEV_1 after inhaled bronchodilator use. The challenge test procedure was as follows. Various concentrations of methacholine in diluent (phosphate-buffered saline), were delivered by a metered nebulizer dosimeter. At each point, FEV_1 was measured. The test was continued until either a reduction of ≥20%in FEV_1 was obtained or the last dose was reached. A PD_{20} <1600 μg was regarded as positive. No individual received β_2-adrenergic receptor agonist administration for at least 24 hours prior to methacholine challenge testing.[46]

2.3. Atopic phenotype: total eosinophil count, total IgE, and specific IgE

Total eosinophil count, the IgE value and specific IgE were measured on blood samples obtained from the asthmatic patients. The IgEs specific for a number of common inhalant allergens in Taiwan, namely, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Dermatophagoides microceras*, cockroach, cat dander and dog dander, were assessed by immunoenzymatic fluorescence assays (IgE-FEIA and RAST-FEIA; Pharmacia CAP system, Uppsala, Sweden). The results were expressed as kU/L, according to the manufacturer's instructions. All the CAP (Capacity) system assays were performed according to previously reports.[14]

2.4. Genotyping

DNA was extracted from blood samples using a commercial kit (QIAamp Blood Kit, Qiagen, Chatsworth, CA, USA).

2.5. IL-4 gene

The C→T mutation 589 base pairs (bp) upstream of the translation start site (GenBank access M23442) in the IL-4 promoter was detected by the loss of a *Bsm* F1 restriction site.[14] A 198-bp fragment was amplified from genomic DNA and then digested with *Bsm* F1 restriction enzyme as described.[14] In the amplification, the 5′ primer used was 5′-TGGGTAAG GACCTTATGGACC3-′ (nucleotides-683 to-663) and the 3′ PCR primer used 5′-GGTGGCATCTTGGAAACTGT-3′ (nucleotides-486 to-505). The fragments measured 198 bp for the (-589T allele) and 120 and 78 bp for the (-589C allele) and were separated on 2% agarose gels.

2.6. ADRβ2 gene

A 219-bp fragment of the B2AR gene was amplified with the following primers designed by using Oligo Primer Analysis Software Version 5 (Molecular Biology Insights, Inc): 5′-AGC CAG TGC GCT TAC CTG CCA GAC T-3′ (sense) and 5′-GCT CGA ACT TGG CAA TGG CTG TGA-3′ (antisense). PCR was performed in a GeneAmp 2400 thermal cycler (Perkin–Elmer) in a reaction volume of 25 μL by using 200 ng of DNA, 1.5 mmol/L $MgCl_2$, 200 mmol/L deoxyribonucleoside triphosphates, 10 pmol of each primer and 0.25 U Taq polymerase (Boehringer Mannheim) in standard PCR buffer (10 mmol/L Tris-HCl [pH 8.3] and 50 mmol/L KCl). PCR involved an initial denaturation at 95 °C for 2 minutes, followed by 35 cycles of denaturation at 95 °C for 40 seconds, annealing at 60 °C for 40 seconds, and extension at 72 °C for 40 seconds, followed by a final extension at 72 °C for 5 minutes. Five microliters of PCR product were electrophoresed on a 2% agarose gel and visualized with ethidium bromide staining and UV illumination to check the PCR amplification.

Allele-specific PCR was performed to assess polymorphisms at nucleic acid 46, which resulted in a change in the encoded amino acid at position 16. Five microliters of primary PCR product were used as the template for allele-specific PCR. The primer pairs (upstream for Arg16, 5′-CTT CTT GCT GGC ACC CTA TA-3′; upstream for Gly16, 5′-CTT CTT GCT GGC ACC CTA TG-3′; downstream, 5′-GGC AAT GGC TGT GAT GAC C-3′) were used to delineate the polymorphism at nucleic acid 46 (amino acid 16). A 151-bp fragment of PCR product was obtained by using the following conditions: 30 cycles of 95 °C for 30 seconds, 62 °C for 50 seconds, and 72 °C for 30 seconds. Five microliters of the PCR product were then electrophoresed on 2% agarose gels.[47]

PCR-restriction fragment length polymorphism was used to assess polymorphisms at nucleic acid 79, which resulted in a change in the encoded amino acid at position 27. PCR reaction was carried out by using 5 μL of primary PCR product as a template. A 139-bp fragment of PCR product was obtained by using the following primers: GCC ATG CGC CGG ACC ACG AC-3′ (sense) and 5′-CGC TCG AAC TTG GCA ATG GCT GTG A-3′ (antisense). Thirty cycles of PCR were carried out at 95 °C for 30 seconds, 65 °C for 30 seconds, and 72 °C for 30 seconds. The PCR products were digested with *Ita*1 restriction enzyme (Roche), run on a 2% agarose gel, and stained with ethidium bromide.[47]

2.7. ADAM33 gene

ADAM33 genes at rs2280091 (T1), rs3918396 (S1), rs3918391 (D1), and rs615436 were analyzed. For all SNPs, primers and probes were obtained from Applied Biosystems TaqMan SNP Genotyping Assays (Nieuwekerk aan de IJssel, The Netherlands). Reactions were performed in 15 μl volumes and contained 10 ng DNA, 1× Taqman Universal Mastermix (FINNZYMES), 200 nM of each probe and 900 nM of each primer. Cycling conditions on the ABI PRISM 7700 HT Sequence Detection System (Applied Biosystems) were 10 min at 95 °C followed by 40 cycles of 15 seconds at 92 °C and 1 minute at 60 °C. End-point fluorescence was measured immediately after cycling. Alleles were assigned using SDS 2.1 software (AppliedBiosystems).

The primary PCR products of different genes were sequenced with the ABI Prism 3700 sequencer (Applied Biosystems) to confirm genotypes' detection in 30 randomly selected products from each genotype.

2.8. Sensitivity and specificity for asthmatic diagnosis by combination of three gene genotypes

Risk genotypes were defined as IL-4 promoter (TT), ADRβ2 (CC), and ADAM33 (AA). Combination genotypes of three genes containing one, two or three risks were studied between the normal and asthma groups.

Sensitivity and specificity was calculated as follows: Sensitivity = number of asthmatic patients with genetic type of one, two or three risk homozygous SNP which associated with asthma/total number of asthmatic patients; Specificity = total number of normal control individuals − number of same genetic type of risk homozygous SNP as

asthmatic patients in normal individuals/total number of normal control individuals

2.9. Statistical analysis

The values for FEV_1%, methacholine dose, total IgE, and total eosinophils count were expressed as the mean ± SD. The frequency genotypes were expressed as number and percentage of the total number. The correlation between gene polymorphism and asthma or its phenotypes was examined by Fisher's exact test, the χ^2 test and the Pearson χ^2 test. An ANOVA test was used to compare the values for FEV_1 %, methacholine dose, total IgE, and total eosinophil count across the three genotypes. Logistic regression was used to evaluate the synergistic and interactive effects of these three genes on asthma.

3. Results

3.1. Correlation between SNPs of the IL-4 promoter genotype, ADRβ2, or ADAM33 genes and asthma

The T allele of the C-589T IL-4 promoter genotype, the C allele of ADRβ2 at Gln27Glu locus and the A allele of the gene of ADAM33 at locus on rs2280091 (T1) were significantly higher in asthmatic sufferers than in normal group (Table 1). However, no association between asthma and SNP at amino acids 16 or haplotypes of the ADRβ2 gene was found. We also found no polymorphisms in ADAM33 at locus rs3918396 (S1), rs3918391 (D1) and rs615436 in the Taiwanese people studied.

Table 1
IL-4 promoter, ADRβ2 or ADAM33 associated with asthma.

Genotype	Control (non-asthma)	Asthma	Odds ratio	95% CI
IL-4 promoter (C-589T locus)	106	452	1.6528	1.1419–2.3922
TT	65 (61.3%)	329 (72.7%)		
TC	34 (32.1%)	110 (24.4%)		
CC	7 (6.6%)	13 (2.9%)		
T allele frequency	0.77	0.85		
C allele frequency	0.23	0.15		
p	0.031			
ADRβ2 (Gln27Glu locus)	115	476	1.5687	1.0118–2.4322
CC	85 (73.9%)	400 (84.0%)		
CG	29 (25.2%)	66 (13.9%)		
GG	1 (0.9%)	10 (2.1%)		
C allele frequency	0.87	0.91		
G allele frequency	0.14	0.09		
p	0.009			
ADAM33 (rs2280091, T1)	115	382	1.9336	1.2064–3.0991
AA	88 (76.5%)	329 (86.1%)		
AG	24 (20.9%)	51 (13.4%)		
GG	3 (2.6%)	2 (0.5%)		
A allele frequency	0.87	0.93		
G allele frequency	0.13	0.07		
p	0.017			

Values are presented for the three genotypes as a percentage of total number, *n* (%), in the group. A = adenine; C = cytosine; CC = allele with cytosine–cytosine homozygote; CI = confidence interval; CT = allele with cytosine–thymine heterozygote; G = guanine; OR = odds ratio; T = thymine; TT = allele with thymine–thymine homozygote. The *p* values are presented for the genotypes of asthma and control groups; OR and CI are presented for the allele frequency of asthma and control groups.

3.2. Correlation between SNP of the IL-4 promoter, ADRβ2 or ADAM33 genes and other phenotypes of asthma

(Tables 2–7) Among asthmatic patients, we found significantly higher eosinophil counts or a lower PD_{20} dose of methacholine challenge to be associated with the A allele, compared to the G allele, of ADAM33 at locus on rs2280091, but there was no significant difference in total IgE and FEV_1 (Table 4). There was no significant difference of FEV_1, total eosinophil count, total IgE, and PD_{20} dose of methacholine challenge in SNPs of C-589T, IL-4 promoter or ADRβ2 at Gln27Glu locus of asthmatic patients (Tables 2 and 3). Comparing the allele of polymorphism in these three genes in asthmatic patients, we found no difference between asthma with allergy to common inhalation antigens (mite, cockroach, dog dander, or cat dander) and asthma without allergy to these antigens (Tables 5–7).

In both control participants and asthmatic patients, allele frequency of SNPs of the three candidate asthma genes was not significantly different in relation to either sex or smoking habit (male vs. female or non-smoker vs. smoker).

3.3. Interaction of these three genes

(Tables 8 and 9) Only 377 asthmatic patients and 106 control participants were completely studied in respect to these three genes. Interaction of these three genes was found to have an additive effect on the risk of asthma (OR: 3.46; CI: 1.51–7.93). Compared with any single risk factor gene, the combined risks of three gene polymorphism with genotypes tended to increase diagnostic sensitivity and specificity (Table 9).

Table 2
Various genotypes of IL-4 promoter polymorphism did not associate with mean values of different phenotypes.

Genotype	*N*	Mean ± SD	95% CI for M	*p*
FEV_1 (% predict)				
TT	293	84.27 ± 52.73	66.61–98.76	0.67
TC	106	75.59 ± 17.81	76.15–83.02	
CC	13	82.69 ± 26.94	78.20–90.32	
Eosinophils (cells/mm^3)				
TT	248	255.40 ± 286.33	219.59–291.21	0.81
TC	82	265.27 ± 240.73	212.37–318.16	
CC	13	212.62 ± 183.67	101.62–323.61	
Methacoline (PC20, mg/mL)				
TT	241	0.91 ± 0.89	0.80–1.02	0.09
TC	77	1.12 ± 0.95	0.91–1.34	
CC	11	1.33 ± 0.96	0.68–1.98	
IgE (KU/mL)				
TT	278	330.29 ± 861.96	228.52–432.06	0.31
TC	93	582.44 ± 2357.20	96.98–1067.90	
CC	13	368.28 ± 574.03	21.40–715.17	

CI = confidence interval of mean; FEV_1 = forced expiratory volume at first and second breath; M = mean; N = number; SD = standard deviation.

Table 3
Various genotypes of ADRβ2-(Gln27Glu locus) did not associate with mean values of different phenotypes.

Genotype	*N*	Mean ± SD	95%CI for M	*p*
FEV_1 (% predict)				
CC	365	83.10 ± 48.16	78.14–88.05	0.88
CG	58	80.72 ± 17.82	75.59–84.96	
GG	7	78.86 ± 16.71	63.39–94.32	
Eosinophils (cells/mm^3)				
CC	305	251.14 ± 273.29	220.34–281.93	0.95
CG	52	248.04 ± 230.83	183.77–312.30	
GG	5	288.00 ± 297.86	81.84–657.84	
Methacoline (PC20, mg/mL)				
CC	294	0.98 ± 0.92	0.88–1.09	0.61
CG	45	1.01 ± 0.96	0.73–1.30	
GG	6	1.35 ± 0.95	0.36–2.35	
IgE (KU/mL)				
CC	339	375.73 ± 1395.79	226.615–524.85	0.97
CG	57	433.50 ± 1102.50	140.87–725.93	
GG	5	207.22 ± 89.44	96.17–318.27	

CI = confidence interval of mean; FEV_1 = forced expiratory volume at first and second breath; M = mean; *N* = number; SD = standard deviation.

4. Discussion

4.1. Correlation between SNPs of the IL-4 promoter, ADRβ2 or ADAM33 genes and other phenotypes of asthma

The current study confirmed that there is an association between the IL-4 promoter polymorphism C-589T and asthma. Furthermore, this study indicated a possible involvement of a single nucleotide polymorphism in the IL-4 gene in the development of asthma, which is consistent with previous studies in the USA,[48] Japan,[16] and Germany,[49] but contrasts with the findings from the UK,[50] Kuwait,[51] and China.[52] The Taiwanese and Chinese populations mainly comprise the same ethnic group, but there are still some minor differences in ethnic composition. Studies focusing on IL-4 promoter gene polymorphism are still controversial in China.[53] The discrepancy between studies may be due to ethnic composition, sample size, the extent to which participants were "truly asthmatic" or differences in control groups.

For IL-4 C-589T , the binding of a transcription factor increases with the presence of the polymorphic T allele.[53] In the light of association studies, it can be hypothesized that this SNP may lead to over expression of the IL-4 gene and thus increase the strength of any IL-4-dependent immunological reaction.

Our results showed a significantly higher C allele frequency of the ADRβ2 genotype at base 79 in asthmatic patients than in normal individuals. Our findings support a UK study showing that glutamine 27 polymorphism is associated with asthma.[29] Glu27 homozygous ADRβ2 was related to hyperbronchial reactivity of asthmatics in the Beijing region.[30] However, in different locus SNPs of the ADRβ2 gene in the USA, ADRβ2 polymorphism at Gly16 allele and body mass index were associated with adult-onset asthma in sedentary but not in active women.[54] In Mexico, there was an association between ADRβ2 at Gly 16 allele and asthma.[24] Conversely, In the UK, no association was noted between ADRβ2 polymorphism and asthma.[32] Moreover, a genetic linkage study in the USA found no linkage at all between asthma and the ADRβ2 gene.[55]

We found that polymorphism of the ADAM33 gene is associated with asthma and further modifies the phenotypes of asthma (severity of hyperresponsiveness and atopy). Therefore, we suggest that the ADAM33 gene may be an important gene for asthma development and remodeling processes. To our knowledge, our study is the first report of an association between ADAM33 polymorphism and eosinophilia in patients with asthma. However, the underlying mechanism is unclear and needs further investigation.

ADAM33 is expressed in airway smooth muscle, fibroblasts, and myofibroblasts, and plays a role in cell signaling, adhesion and proteolysis.[56] Interestingly, the existence of various ADAM33 isoforms in human embryonic bronchi and their surrounding mesenchyme indicates its contribution to smooth muscle development and function. Additionally, its presence in mesenchymal tissues is thought to cause ''unusual'' airway formation that leads to the origins of asthma in early life. Thus, the SNP potentially modulating the catalytic domain of ADAM33 could contribute to asthma pathogenesis. The association of ADAM33 with asthma phenotypes as a "susceptibility gene" has been replicated in several cohorts, and genetic variation in this gene has been linked to accelerating decline in lung function.[57] Recently, Lee and colleagues applied this genetic knowledge to cell biology and measured ADAM33 levels in bronchoalveolar lavage fluid and endobronchial biopsies from patients with asthma.[58] They found a correlation between higher ADAM33 levels and increased chronic airflow obstruction. ADAM33 has become not just an asthma susceptibility gene, but also a biomarker for disease severity, playing a complex cause-and-effect role for airway remodeling in asthma.

Table 4
Various genotypes of ADAM33 (rs2280091) associated with different phenotypes.

Genotype	*N*	Mean ± SD	95% CI for M	*p*
FEV_1 (% predict)				
AA	294	82.59 ± 52.51	76.57–88.62	0.95
AG	45	83.39 ± 20.85	77.13–89.66	
GG	2	93.50 ± 4.95	49.03–137.97	
Eosinophils (cells/mm^3)				
AA	236	254.61 ± 256.17	212.75–278.46	0.028
AG	36	373.58 ± 423.69	230.23–516.94	
GG	2	75.00 ± 35.36	242.66–392.66	
Methacoline (PC20, mg/mL)				
AA	225	0.92 ± 0.90	0.80–1.04	0.038
AG	36	0.86 ± 7.48	0.60–1.11	
GG	2	2.50 ± 0	2.50–2.50	
IgE (KU/mL)				
AA	269	370.29 ± 969.08	253.96–486.62	0.85
AG	43	295.97 ± 684.80	85.22–506.72	
GG	2	151.5 ± 36.06	172.51–475.51	

CI = confidence interval of mean; FEV_1 = forced expiratory volume at first and second breath; M = mean; N = number; SD = standard deviation.

Table 5
Comparison between frequency of the genotypes of the IL-4 gene promoter for normal controls and asthmatic patients with various allergy and non-allergy phenotypes.

Groups	Genotypes					Allele frequency	
	TT	CT	CC	T	C	*p1*	*p2*
Normal	65 (61.3%)	34 (30.4%)	7 (6.3%)	0.77	0.23		
Asthma							
Allergy to mite							
$n = 231$	159 (68.8%)	67 (29.0%)	5 (2.2%)	0.83	0.17	0.09	
Non-allergy to mite							
$n = 171$	124 (72.5%)	41 (24%)	6 (3.5%)	0.85	0.15	0.13	0.41
Allergy to cockroach							
$n = 106$	73 (68.9%)	32 (30.2%)	1 (0.9%)	0.84	0.16	0.08	
Non-allergy							
$n = 296$	210 (70.9)%	76 (25.7%)	10 (3.4%)	0.84	0.16	0.13	0.31
Allergy to dog dander							
$n = 87$	66 (75.9%)	21 (24.1%)	0 (0%)	0.88	0.12	0.02	
Non-allergy							
$n = 315$	217 (68.9%)	87 (27.6%)	11 (3.5%)	0.83	0.17	0.22	0.15
Allergy to cat dander							
$n = 31$	24 (77.4%)	6 (19.4%)	1 (3.2%)	0.87	0.13	0.25	
Non-allergy							
$n = 371$	259 (69.8%)	102 (27.5%)	10 (2.7%)	0.84	0.16	0.08	0.62
High total Ig E							
$n = 136$	102 (75%)	32 (23.5%)	2 (1.5%)	0.87	0.13	0.03	
Normal total Ig E							
$n = 215$	161 (74.9%)	47 (21.9%)	7 (3.2%)	0.86	0.14	0.04	0.57
High total eosinophil count							
$n = 47$	35 (74.5%)	10 (21.3%)	2 (4.3%)	0.85	0.15	0.29	
Normal ≤450/ml							
$n = 278$	207 (74.5%)	62 (22.3%)	9 (3.2%)	0.86	0.14	0.03	0.93

Values are presented in the three genotypes as percentage of total number, *n* (%), in the group. Pearson's χ^2 test was used to compare the two groups (normal vs. asthma with allergy; normal vs. asthma without allergy; asthma with allergy vs. asthma without allergy); To compare three genotypes, *P1*: normal versus asthma with allergy or normal versus asthma non-allergy; *P2*: asthma with allergy versus asthma without allergy.

Table 6
Comparison between frequency of the genotypes of the ADRβ2 (Gln27Glu locus) gene promoter for normal control and asthmatic patient with various allergy and non-allergy phenotypes.

Groups	Genotypes					Allele frequency	
	CC	CG	GG	C	G	*P1* value	*P2* value
Normal	85 (73.9 %)	29 (25.2%)	1 (0.9%)	0.87	0.13		
Asthma							
Allergy to mite							
$n = 237$	198 (83.5%)	36 (15.2%)	3 (1.3%)	0.91	0.09	0.07	
Non-allergy to mite							
$n = 183$	158 (86.3%)	22 (12%)	3 (1.6%)	0.92	0.08	0.01	0.62
Allergy to cockroach							
$n = 108$	89 (82.4%)	19 (17.6%)	0 (0%)	0.91	0.09	0.23	
Non-allergy							
$n = 312$	267 (85.6%)	39 (12.5%)	6 (2%)	0.92	0.08	0.01	0.16
Allergy to dog dander							
$n = 88$	72 (81.8%)	15 (17%)	1 (1.1%)	0.90	0.10	0.37	
Non-allergy							
$n = 332$	284 (85.5%)	43 (13%)	5 (1.5%)	0.92	0.08	0.08	0.60
Allergy to cat dander							
$n = 31$	26 (83.9%)	5 (16.1%)	0 (0%)	0.92	0.08	0.48	
Non-allergy							
$n = 389$	330 (84.8%)	53 (13.6%)	6 (1.5%)	0.92	0.08	0.01	0.74
High total Ig E							
$n = 147$	126 (85.7%)	20 (13.6%)	1 (0.7%)	0.93	0.07	0.06	
Normal total Ig E							
$n = 260$	218 (83.8%)	37 (14.2%)	5 (1.9%)	0.91	0.09	0.03	0.59
High total eosinophil count							
$n = 48$	42 (87.5%)	5 (10.4%)	1 (2.1%)	0.93	0.07	0.09	
Normal ≤450/ml							
$n = 316$	267 (84.5%)	45 (14.2%)	4 (1.3%)	0.92	0.08	0.03	0.71

Values are presented in the three genotypes as percentage of total number, n (%), in the group. Pearson's χ^2 test was used to compare the two groups (normal vs. asthma with allergy; normal vs. asthma without allergy; asthma with allergy vs. asthma without allergy); To compare three genotypes, *P1*: normal versus asthma with allergy or normal versus asthma non-allergy; *P2*: asthma with allergy versus asthma without allergy.

Table 7
Comparison between frequency of the genotypes of the ADMA33 gene at rs2280091 (T1) for normal controls and asthmatic patients with various allergy and non-allergy.

Groups	Genotypes					Allele frequency	
	AA	AG	GG	A	G	*p1*	*p2*
Normal $n = 115$	88 (76.5%)	24 (20.9%)	3 (2.6%)	0.87	0.13		
Asthma							
Allergy to mite							
$n = 196$	167 (85.2%)	18 (14.3%)	1 (0.5%)	0.92	0.08	0.06	
Non-allergy to mite							
$n = 131$	116 (88.5%)	14 (10.7%)	1 (0.8%)	0.94	0.05	0.04	0.61
Allergy to cockroach							
$n = 89$	77 (86.5%)	12 (13.5%)	0 (0%)	0.93	0.07	0.11	
Non-allergy							
$n = 238$	206 (86.6)%	30 (12.6%)	2 (0.8%)	0.89	0.11	0.047	0.67
Allergy to dog dander							
$n = 59$	52 (88.1%)	7 (11.9%)	0 (0%)	0.95	0.05	0.14	
Non-allergy							
$n = 268$	231 (86.2%)	35 (13.1%)	2 (0.7%)	0.93	0.07	0.04	0.77
Allergy to cat dander							
$n = 41$	37 (90.4%)	4 (9.8%)	0 (0%)	0.95	0.05	0.15	
Non-allergy							
$n = 286$	246 (86.0%)	38 (13.3%)	2 (0.3%)	0.93	0.07	0.04	0.70
High total Ig E							
$n = 113$	100 (88.5%)	13 (11.5%)	0 (0%)	0.94	0.06	0.03	
Normal total Ig E							
$n = 202$	171 (84.7%)	29 (14.4%)	2 (3.2%)	0.92	0.08	0.16	0.43
High total eosinophil count							
$n = 38$	31 (81.6%)	6 (15.8%)	1 (2.6%)	0.89	0.11	0.79	
Normal ≤450/ml							
$n = 237$	209 (88.2%)	27 (11.4%)	1 (0.4%)	0.94	0.06	0.01	0.24

Values are presented for the three genotypes as percentage of total number, *n* (%), in the group. Pearson's $\chi 2$ test was used to compare the two groups (normal vs. asthma with allergy; normal vs. asthma without allergy; asthma with allergy vs. asthma without allergy); To compare three genotypes, *P1*: normal versus asthma with allergy or normal versus asthma non-allergy; *P2*: asthma with allergy versus asthma without allergy.

Our study supports previous results that SNPs of ADAM33 was associated with asthma. However, it was found at different SNP loci in people from different countries. ADAM33 associated with asthma was found in the USA at I1,L-1, M+1, T1,T2, T+1 SNP,[10] and in the UK at F+1, Q-1,S1, S2, ST+4, V-1, V4.[10] An association between asthma and ADAM33 SNP at ST+7 and V4 was found in Dutch, S2 in African Americans, ST+7, T1 and T2 in whites and S2, T1 and T2 in Hispanics[39]. SNP of ADAM 33 was associated with asthma at ST+7 in Germans.[40] T1 and haplotype were associated with hyperresponsiveness in Koreans.[43] Two SNPs in strong linkage disequilibrium (T1 and T+1) were marginally associated with asthma in the Hispanic cohort.[45] Minor alleles of S11, ST14, and T2 SNPs were over-transmitted to asthma-affected offspring in Japan .[39] In contrast, ADAM33 was not associated with asthma in Puerto Rican or Mexican populations .[41] There was no significant association between three SNPs of ADAM33 (7575G/A in intron 6, 11188A/T in intron 19, and 12433T/C in exon 20) and asthma susceptibility in the Chinese population.[47] Furthermore, no single SNPs (F+1, M+1, S1, S2, ST+4, ST+5, ST+7, T1, T2 and V4) had any significant association with asthma in the German population Similarly, no significant association between any of the 10 SNPs (F+1, Q-1, S1, ST+4, ST+7, V-2, V-1, V2, V4, V5) and asthma was found in Australia.[36]

4.2. Interaction of IL-4 ADRβ2 and ADAM 33

Asthma contains phenotypes such as bronchoconstriction, airway remodeling, hyperactivity, mucus hypersecretion, and persistent inflammation. These complex traits might be caused by different and multiple genes. Our results are the first to show that the interaction of these three genes has an additive effect on the risk of asthma and they demonstrate that

Table 8
The interaction of three genes (IL-4, ADRβ2, and ADAM 33) in the same population and ethnic group with 377 asthmatic patients and106 control subjects.

Genotypes IL4/ADRβ2/ADAM 33	No.	OR	95% CI	*p*
11/11/11	29	1 (asthma: 14; control: 15)		
12/12/12	5	2.44 (asthma:4; control: 1)	0.24–24.78	0.449
12/12/22	41	1.67 (asthma:25; control: 16)	0.60–4.62	0.326
12/22/22	168	1.57 (asthma:103; contol: 65)	0.69–3.58	0.280
22/22/22	240	3.46 (asthma:186; control: 54)	1.51–7.93	0.003

No.: number; OR: Odd Ratio; CI: confidence interval; 11: no risk allele; 22: risk alleles.

Table 9
Sensitivity or specificity of combination with various risk genotypes of three genes, IL-4, ADRβ2, and ADAM 33, in the same population and ethnic group with 377 asthmatic patients and 106 control subjects.

Groups	Genotypes	Asthma *N*	Normal *N*	Sensitivity	Specificity
3 risks	TTCCAA	204	36	0.54	0.66
2 risks	TTCCAG	27	15		
	TTCCGG	2	0		
	TTCGAA	34	9		
	TTGGAA	7	1		
	TCCCAA	60	23	0.36	0.51
	CCCCAA	7	4		
1 risk	TTCGAG	6	2		
	TTCGGG	0	2		
	TTGGAG	1	0		
	TTGGGG	0	0		
	TCCCAG	13	4		
	TCCCGG	0	0		
	CCCCAG	0	2		
	CCCCGG	0	0		
	TCCGAA	11	5		
	TCGGAA	1	0		
	CCCGAA	0	1		
	CCGGAA	0	0	0.09	0.84
<1 risk	TCCGAG	4	1		
	CCGGGG	0	0		
	TCCGGG	0	1		

A = adenine; C = cytosine; CC = allele with cytosine−cytosine homozygote; CT = allele with cytosine−thymine heterozygote; G = guanine; *N* = number; T = thymine; TT = allele with thymine−thymine homozygote.

a combination of the three genetic polymorphisms produces an increase in the sensitivity and specificity for genetic diagnosis of asthma. This finding supports the view that asthma is a complex polygenic disease and that more combined genes are needed to predict the risk of asthma and to improve the accuracy of genetic diagnosis of asthma. Based on our findings, each candidate gene might modulate a weak association with asthma. but a combination of more genes that modulate the different role of pathogenesis, such as IL4 for persistent inflammation, ADRβ2 for bronchodilatation and ADAM33 for airway remodeling and hyperactivity in our study, respectively, have an additive effect on the association with asthma. In the future, it will be necessary to elucidate all genes and their role in asthma to reliably make a genetic diagnosis and predict the risk of asthma.

The results of previous studies of SNPs of IL-4, ADRβ2, or ADAM 33 gene replication have been inconclusive, probably due to inconsistencies in asthma phenotypes, study designs, ethnically diverse populations and the rigidity of criteria for asthmatic enrollment by definite diagnosis or as yet unknown environmental influences.

However, asthmatic patients in the present study were strictly enrolled based on clear diagnostic criteria and received regular long-term follow-up by only one physician at the outpatient clinic of Taipei Veterans General Hospital, Taiwan.

In conclusion, IL-4, ADRβ2 and ADAM 33 are associated with asthma. Furthermore, the ADAM 33 gene is a disease modifier in terms of the severity of airway hyperresponsiveness and eosinophilia. The IL-4 promoter gene is associated with asthmatic patients who have an allergy to dog dander. The combined three genetic polymorphisms have an additive effect on the risk of asthma and genetic diagnosis of asthma. Asthma is a complex polygenic disease, which will demand a greater number of combined genes to improve the genetic diagnosis of asthma and predict the risk of the disorder.

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